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A. U. Uebing-Czipura [a]; H. D. Dawson [b]; M. S. Rutherford [c]; G. Scherba [a]
[a] Department of Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana, IL, USA [b] Diet, Genomics and Immunology Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD, USA [c] Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN, USA

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TRANSCRIPTOME PROFILE AND CYTOGENETIC ANALYSIS OF IMMORTALIZED NEURONALLY RESTRICTED PROGENITOR CELLS DERIVED FROM THE PORCINE OLFACTORY BULB

A. U. Uebing-Czipura[1], H. D. Dawson[2], M. S. Rutherford[3], and G. Scherba[1]

[1]*Department of Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana, IL, USA*

[2]*Diet, Genomics and Immunology Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD, USA*

[3]*Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN, USA*

Recently, we established and phenotypically characterized an immortalized porcine olfactory bulb neuroblast cell line, OBGF400 (1). To facilitate the future application of these cells in studies of neurological dysfunctions and neuronal pathogen interactions, a comprehensive knowledge of their genomic variability and overall gene expression capacity was pursued. Accordingly, the OBGF400 cells were subjected to karyotyping and more extensive transcriptome analyses. Cytogenetic characterization of these cells revealed a genetic mosaicism of neuronal hyperdiploidy. A direct comparison of the OBGF400 cell transcriptome pattern, generated by utilizing the Affymetrix GeneChip® Porcine Genome Array, to that of a non-neural, porcine epithelial cell line facilitated the identification of 831 probe sets preferentially hybridized by the neuroblast transcripts. Subsequent functional annotation of these OBGF400 RNAs using the Database for Annotation, Visualization and Integrated Discovery 2008 enabled their allocation to the corresponding gene ontology biological process term, thereby assisting the recognition of key elements involved in the regulation of neuronal signal transduction and neurogenesis.

Keywords: Cytogenetic analysis; Neuronal aneuploidy; Porcine neuronal progenitor cell line; Transcriptome pattern

The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (Edgar et al., 2002) and are accessible through GEO Series accession number GSE16855 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE16855).

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Address correspondence to G. Scherba, Department of Pathobiology, University of Illinois, 2001 South Lincoln Avenue, Urbana, IL 61802, USA. E-mail: scherba@illinois.edu

INTRODUCTION

The isolation and *in vitro* cultivation of neurons with subsequent genotypic and phenotypic characterization are the first steps in the process of establishing an *in vitro* neuronal disease model system. In this regard, the olfactory bulb (OB) core represents an excellent source of lineage-committed neuroblasts, as it is known to harbor both multi-potent progenitor cells and lineage-restricted neuroblasts in neonatal and adult animals (2). The latter population originates in the subventricular zone (SVZ) and migrates tangentially by way of the rostral migratory stream (RMS) to the core of the OB. Contrary to the central dogma, although these neuronally restricted progenitor cells display mature neuronal cell characteristics, they do not become senescent (3). This unique attribute not only facilitates their mitotic expansion for *in vitro* studies, but also favors its potential application in regenerative therapies.

To develop a continuous population of such mitotic neurons, we previously isolated porcine OB-derived neuroblasts and subsequently immortalized them by the insertion of a transcriptional unit, designed to regulate the expression of a linked human telomerase catalytic subunit (hTERT) cDNA, into their cellular chromosomes (1). A resulting subclone, designated as OBGF400, demonstrated continuous mitotic competency while their non-transduced counterparts terminally differentiated and succumbed rapidly in culture. Phenotypic characterization of this novel cell line, based on transcription patterns and verified by immunocytochemical detection of relevant proteins, demonstrated its unique commitment to the neuronal lineage. During continuous subpassaging, hTERT RNA levels, as well as the extent of telomerase activity, remained relatively constant. Likewise, the levels of transcripts encoding the tumor suppressor protein p53 and proto-oncogene c-myc (a marker for tumorigenesis and neoplastic transformation) were unchanged. Moreover, the OBGF400 cells maintained karyotypic stability during subpassaging with the majority being in the G0/G1 phase of the cell cycle with an overall lack of cellular senescence.

Intriguingly, karyotypic abnormalities in neuronal cells derived from the developing and mature mammalian central nervous system (CNS) recently have been discovered and were not associated with tumorigenic disease processes (4, 5). These observations and the future application of the OBGF400 neuroblasts necessitated their further genotypic characterization by cytogenetic analysis. Moreover, the initial characterization of the OBGF400 cells considered the expression of only 11 neural-specific genes (1). Clearly a more exacting survey is necessary before these cells can be used in studies involving neurological issues and neuron-pathogen interactions. Therefore, we extended the previous transcriptional phenotyping through the application of a recently available porcine-specific microarray chip and obtained a whole genome-scaled expression profile of the neuronally committed progenitor cells. Subsequent comparison of the array results to those of the non-neural, porcine kidney epithelial cell line, PK15, enabled the identification of transcripts unique to the OBGF400 cells. Functional annotation of the OBGF400 cellular transcriptome pattern delineated potential signal transduction and neurogenic processes not previously recognized in such neuronally restricted precursors.

MATERIALS AND METHODS

Propagation of OBGF400 and PK15 Cells

OBGF400 cells were maintained as previously described (1). PK15 cells were propagated in minimum essential medium (MEM) (Sigma) supplemented with 10% bovine calf serum (Hyclone, Logan, UT), 100 U/ml penicillin (Sigma), 0.1 mg/ml streptomycin (Sigma), 0.05 mg/ml gentamicin (Sigma) and 0.0025 mg/ml amphotericin B (Sigma).

Karyotype Analysis of OBGF400 Cells

Karyotype analysis was performed on three independent harvests of OBGF400 cells (estimated 60 population doublings) at the Cytogenetics Facility of the University of Pittsburgh Cancer Institute (Pittsburgh, PA). Chromosome spreads were obtained after mitotically arresting the OBGF400 cells by a standard protocol (6). Metaphase profile analysis based on trypsin-Giemsa banding was performed on a total of thirteen OBGF400 cells. Chromosomes were arranged based on the pig chromosome complement designation by the Roslin Institute (Roslin BioCentre Midlothian, UK): www.thearkdb.org/arkdb/do/getChromosomeDetails?accession=ARKSPC00000001.

RNA Extraction and Microarray Hybridization

OBGF400 and PK15 cells were seeded into nine 75-cm^2 flasks (Midwest Scientific, St. Louis, MO) each at a density of 1×10^3 cells/flask and incubated until confluent. Total RNA was independently isolated from each monolayer by using a RNeasy Mini kit (Qiagen, Valencia, CA) as recommended by the manufacturer. After RNA extraction, all samples were DNase-treated at 37°C for 20 min with TURBO DNase (Ambion, Austin, TX). In lieu of the DNase inactivation step, immediate sample purification and concentration were performed with RNeasy Clean-Up Columns (Qiagen). Prior to the purification step, the individual extracts were pooled in sets of three to obtain a total of three biologically independent RNA samples per cell type. Concentrations and purity of the pooled RNA preparations were determined using the BioAgilent RNA assay (Agilent Technologies, Inc., Santa Clara, CA).

Microarray analysis of the RNA samples was performed at the W. M. Keck Center for Comparative and Functional Genomics (Roy J. Carver Biotechnology Center, University of Illinois, Urbana, IL). The six biological replicates were processed and hybridized to separate Affymetrix GeneChip® Porcine Genome Expression Arrays (Affymetrix, Inc., Santa Clara, CA) utilizing the GeneChip® Expression 3′-Amplification Reagents One-Cycle Target Labeling and Control Reagents kit as well as the GeneChip® Sample Cleanup Module (Affymetrix, Inc.) according to the manufacturer's instructions. Following hybridization, each chip was washed, incubated with streptavidin-conjugated phycoerythrin dye (Invitrogen Corp., Carlsbad, CA), followed by stain enhancement with biotinylated goat anti-streptavidin antibody (Vector Laboratories, Burlingame, CA) utilizing a

GeneChip® Fluidics Station 450 and the GeneChip® Operating Software version 1.1 (Affymetrix, Inc.). The hybridization fluorescence signal intensities were determined by using a GeneChip® Scanner 3000 (Affymetrix, Inc.) and then the data was subjected to quality control assessments prior to statistical analysis executed by the Bioinformatics Unit of the W.M. Keck Center.

Statistical Analysis and Functional Annotation

The Affymetrix GeneChip® Porcine Genome Expression Array contains 24,123 probe sets based on DNA sequences from UniGene Build 28 (August 2004) and other porcine RNA sequences in GenBank® up to August 24, 2004. Each probe set (excluding controls) consists of 22 unique 25-mers: 11 perfect match (PM) probes based on the target DNA and 11 mismatch (MM) probes that have the 13th base changed to provide an estimate of the background fluorescence due to non-specific binding. Spearman rank correlation coefficient was used to assess the consistency of raw probe-level values among the three replicates of the OBGF400 and PK15 cells.

Affymetrix originally estimated that the 24,123 probe sets represented 20,201 *S. scrofa* genes; however Tsai et al. (7) re-annotated the array by BLASTing Affymetrix's target sequences (extended based on TIGR assembly, if possible) against Ensembl's human cDNA sequence library; this newer annotation data contains 12,124 unique gene names.

To characterize the transcriptome of the OBGF400 cells, we categorized each probe set as present, marginal or absent (PMA calls) on each array based on the Affymetrix detection call algorithm (8) as implemented in the Affy package (9) from the Bioconductor project (10). The default values were used for threshold Tau (0.015) and p-value cut-offs for absent ($p > 0.06$), marginal ($p < 0.06$), and present ($p < 0.04$). The detection call algorithm compares each PM probe in a probe set to its corresponding MM probe and calls it "larger" than the MM if the proportional increase in PM is above some threshold ($Tau = 0.015$). The algorithm then performs a Wilcoxon signed-ranks test to see if more PM probes are larger than MM probes. Hence, the "present" call is a statistically derived threshold reflecting a relative confidence that the desired mRNA is indeed present at a level significantly above background hybridization. An "absent" call, however, indicates that either the target mRNA was not present or that non-specific cross-hybridization has occurred.

To establish probe sets that were preferentially hybridized by the OBGF400 transcripts but not by those from the PK15 cells, we compared PMA calls between the OBGF400 and PK15 arrays; however, as batch effects are confounded with the individual cell types, differences in expression levels between the two cell types could not be assessed.

To validate our methodology for determining probe sets that were "uniquely" identified, we applied our detection call criteria to a previously published study (11). In this study, the investigators prepared a sample containing 3,860 of the 14,010 known RNA species on the Affymetrix Drosophila Genome Array (Drosgenome1) to determine the accuracy of the Affymetrix detection call algorithm, which achieved 85% accuracy before exceeding a 10% false-positive rate for a single array. When we applied our criteria of present detection calls to this published data, 87.6% of the

truly present probe sets were identified while only 9.8% were false positives. This validation demonstrated that our methodology was capable of detecting the vast majority of truly hybridized probe sets with only a relatively small percentage of false positives.

Functional annotation clustering as well as the identification of biological process ontologies was performed on probe sets preferentially hybridized by the OBGF400 cellular transcripts using the web-based Database for Annotation, Visualization and Integrated Discovery (DAVID) 2008 (12) software. DAVID provides minimal support for porcine genes, therefore the equivalent human Affymetrix probe set IDs from the Tsai et al. (7) annotation were imported into the software program. The gene list was annotated at a medium stringency level to ensure a relatively high specificity. The resultant annotation clusters were ranked based on their biological significance by using the group enrichment score, which consists of the geometric mean (in log-scale) of the annotation member's p-values in a corresponding cluster. Furthermore, analogous p-values and the Benjamini statistic for multiple correction comparison (13) were provided, with the latter controlling the false discovery rate of family-wise enriched terms.

RESULTS

Karyotype Analysis of OBGF400 Cells

To determine the karyotypic character of the cultured hTERT-immortalized OBGF400 neuroblasts (obtained from a female newborn), cytogenetic preparations of 13 trypsin-Giemsa banded metaphase cells were analyzed. In comparison to a normal pig karyotype with 38 chromosomes, the OBGF400 cell chromosome numbers ranged from 40 to 47 and, thus, these cells were hyperdiploid (Figure 1) and demonstrated numerical chromosomal instability (three copies of chromosomes 1, 2, 12, 17, and 18 were observed as clonal aberrations since each was seen in two or more cells). Each of the 13 examined cells had one or two copies of an X chromosome. Five cells also contained a small, unidentified chromosome. Although several non-clonal changes such as the random loss of one or more copies of different chromosomes were observed, this most likely could be attributable to the slide making process and, thus, were considered a technical artifact.

Transcriptome Analysis of the Neuronally Restricted Progenitor Cells

Utilization of the Affymetrix GeneChip® Porcine Genome Expression Array enabled the first whole genome-scale expression profile to be established for the OBGF400 cell line and swine neuronal cells in general. While high-throughput procedures such as microarrays represent an excellent tool for establishing whole genome-scaled datasets, their accuracy and relative performance is often questioned. Consequently, in addition to Affymetrix's recommended quality control assessment, we performed further analyses as implemented in the affyPLM (14) and Affy (9) packages from the Bioconductor project. These assessments (data not shown) and the Spearman rank correlation coefficients for the raw (e.g., without background

Figure 1 Karyotype analysis of the OBGF400 neuroblasts. This representative metaphase chromosome spread shows aneuploidy with a preponderance of hyperdiploidy in the OBGF400 cells. Numerical abnormalities for chromosomes 1, 14, 17, and 18 are apparent. The unlabeled small chromosome has not been identified.

correction or normalization) probe-level values (Table 1) determined that all arrays were of high quality and extremely consistent within each cell type.

The total number of PMA calls was determined for each of the three biologically independent array replicates of each cell type (Table 2). Based on the Affymetrix detection call algorithm, 12,687 and 12,204 probe sets were identified as present in all three arrays for the OBGF400 and PK15 cells, respectively. To ascertain genes preferentially expressed by the OBGF400 neuroblasts (to the exclusion of PK15 somatic cell background), we identified 831 probe sets that were present ($p < 0.04$)

Table 1 Spearman correlation coefficients (for raw probe-level values) for the three biologically independent OBGF400 and PK15 microarray hybridizations

	OBGF400 cells			PK15 cells		
Correlation of replicates	1 vs. 2	1 vs. 3	2 vs. 3	1 vs. 2	1 vs. 3	2 vs. 3
PM* and MM[f] values	0.9823	0.9820	0.9813	0.9653	0.9732	0.9659
PM values	0.9893	0.9887	0.9888	0.9792	0.9825	0.9794
PM values "only present genes"	0.9924	0.9926	0.9934	0.9863	0.9880	0.9879

*Perfect match probe.
[f]Mismatch probe.

Table 2 Number of present, marginal, or absent probe sets among the three biologically independent OBGF400 and PK15 microarray replicates

	OBGF400 cells			PK15 cells		
Affymetrix detection call	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Present calls	13,753	13,748	13,521	13,449	13,289	13,559
Marginal calls	607	638	651	702	651	683
Absent calls	9763	9737	9951	9972	10,183	9881

in all three OBGF400 replicates but absent ($p > 0.06$) in all three PK15 replicates. These 831 probe sets represent 709 unique gene names, which map to 685 human Affymetrix IDs; DAVID had functional annotation for 644 of these human Affymetrix IDs.

Identification of OBGF400 Genes Potentially Involved in the Establishment of the Neuronal Genetic Mosaicism

The formation of genomic diversity and neuronal mosaicism, at least in part, depends on non-homologous end joining factors, homologous recombination, and molecules essential for DNA double strand repair mechanisms. The whole genome-scaled expression profile of the porcine neuroblasts identified transcripts for several important regulatory factors: DNA repair protein XRCC4, Fanconi anemia group G protein (known as DNA repair protein XRCC9), DNA ligase IV, ATP-dependent DNA helicase II 70 and 80 kDa subunits (KU70 and KU80), and calcium and integrin binding 1 (CIB1) genes. The DNA surveillance genes, ataxia telangiectasia (Rad3-related protein), and tumor suppressor p53, were expressed simultaneously with the base-excision repair protein, DNA polymerase β gene. In addition, genes for two key mitotic regulators, pituitary tumor transforming 1 (securin) and extra spindle pole like 1 (separanase), were transcribed in the dividing OBGF400 neuroblasts.

Functional Annotation Clusters Identified in the OBGF400 Transcriptome Profile

The DAVID functional annotation cluster analysis (medium stringency level) mapped the probe sets inherent to the porcine OBGF400 cells to their corresponding biological process, molecular function, and cellular compartment terms and provided detailed insight into the gene ontology (GO) of the neuroblasts. The analysis yielded a total of 216 annotation clusters; each assigned with a specific group enrichment score as well as the annotation member's p-value and the Benjamini statistic. Clusters 21 and 24 with the corresponding enrichment scores of 2.67 and 2.26, respectively, were selected for downstream evaluation (Tables 3 and 4, respectively). In consideration of the future use of the OBGF400 cells as an *in vitro* neuronal model system, we examined clusters with biological process terms associated with neurogenesis and cell development (referred to as cluster 21, Table 5) as well as terms implicative of signal transduction (referred to as cluster 24, Table 6). Examination of

Table 3 Functional annotation cluster 21 of the gene ontology (GO) biological process (BP) terms identified as relevant for OBGF400 neurogenesis

Annotation cluster 21			
GO term_BP	Count*	p-Value	Benjamini
Generation of neurons	23	6.3E-4	7.1E-2
Neurogenesis	24	7.4E-4	7.5E-2
Neurite development	16	8.7E-4	8.6E-2
Cell morphogenesis	33	8.8E-4	8.3E-2
Cellular structure morphogenesis	33	8.8E-4	8.3E-2
Neuron differentiation	20	1.5E-3	1.2E-1
Cell projection organization	19	1.8E-3	1.4E-1
Cell part morphogenesis	19	1.8E-3	1.4E-1
Cell projection morphogenesis	19	1.8E-3	1.4E-1
Neuron development	16	3.6E-3	2.0E-1
Neurite morphogenesis	13	6.0E-3	2.9E-1
Neuron morphogenesis during differentiation	13	6.0E-3	2.9E-1
Axonogenesis	12	1.0E-3	4.1E-1
Cellular morphogenesis during differentiation	13	1.0E-3	4.0E-1

*Number of genes assigned to the GO term.

these two annotation clusters fostered the neuronal character of the OBGF400 cells as represented by the consistently expressed genes and their individual attribution to the respective GO terms.

The annotation cluster 21 (Table 5), which summarizes the genes involved in neurogenesis as well as cell and neuron morphogenesis, included expression products implemented in guided neuronal cell migration. Regulators of cytoskeletal dynamics, adhesion and migration events comprised the extracellular matrix glycoprotein tenascin N (TNN), the integrin alpha 8 (ITGA8), laminin beta 1 (LAMB1), and the focal adhesion protein vinculin (VCL). Moreover, the integrin regulator ephrin-B2 (EFNB2) was expressed by the OBGF400 neuroblasts; however, it was not represented in the annotation cluster 21 but rather assigned the GO term cell-cell signaling (cluster 24). Likewise, cell dynamic and cell movement regulating genes such as the F-actin binding protein developmentally regulated brain protein drebrin

Table 4 Functional annotation cluster 24 of the gene ontology (GO) biological process (BP) terms identified as relevant for OBGF400 signaling mechanisms

Annotation cluster 24			
GO term_BP	Count*	p-Value	Benjamini
Cell-cell signaling	43	1.8E-4	2.7E-2
Transmission of nerve impulse	25	1.4E-3	1.2E-1
Synaptic transmission	22	2.7E-3	1.7E-1
System process	68	1.6E-2	5.0E-1
Neurological system process	44	4.4E-1	1.0E-0

*Number of genes assigned to the GO term.

Table 5 Consistently expressed genes relevant for neurogenesis as identified by the DAVID functional annotation analysis of the swine neuronal OBGF400 cells

Annotation cluster 21: Neurogenesis-relevant gene ontology (GO) terms		Affymetrix ID		
Gene name	Gene ID	Porcine	Human	GO terms
Adrenergic alpha-1B receptor	ADRA1B	Ssc.17370.1.A1_at	207589_at	Cell- and cell structure morphogenesis
Arachidonate 12 lipoxygenase	ALOX12	Ssc. 4415.1.S1_at	207206_s_at	Cell- and cell structure morphogenesis
Axon guidance receptor (roundabout homolog 1)	ROBO1	Ssc. 12138.1.A1_at	213194_at	Cellular morphogenesis during differentiation Axonogenesis Neuron morphogenesis during differentiation Neurite morphogenesis Neuron development Cell projection morphogenesis- and organization Cell part morphogenesis Generation of neurons and neuron differentiation Neurogenesis Neurite development Cell- and cell structure morphogenesis
CAMP responsive element binding protein 1	CREBP1	Ssc.16173.1.S1_at	204312_x_at	Cellular morphogenesis during differentiation Axonogenesis Neuron morphogenesis during differentiation Neurite morphogenesis Neuron development Cell projection morphogenesis- and organization Cell part morphogenesis Generation of neurons and neuron differentiation Neurogenesis Neurite development Cell- and cell structure morphogenesis
Centaurin, delta 3	LUM	Ssc.1128.1.S1_at	229554_at	Cell- and cell structure morphogenesis
Cyclin-dependent kinase inhibitor 2a	CDKN2A	Ssc.15750.1.S1_at	207039_at	Cell- and cell structure morphogenesis
Drebrin 1 (developmentally regulated brain protein)	DBN1	Ssc.30502.1.A1_at	202806_at	Neuron development Cell projection morphogenesis- and organization

				Cell part morphogenesis Generation of neurons and neuron differentiation Neurogenesis Neurite development Cell- and cell structure morphogenesis
Enolase 1	ENO1	Ssc.10942.1.S1_at	204483_at	Cell- and cell structure morphogenesis
Fibroblast growth factor receptor 1	FGFR1	Ssc.17773.1.S1_at	211535_s_at	Cell- and cell structure morphogenesis
Fibroblast growth factor receptor 2	FGFR2	Ssc.15840.1.S1_at	211401_s_at	Cell- and cell structure morphogenesis
Forkhead box F1	FOXF1	Ssc.2585.2.S1_at	205935_at	Cell- and cell structure morphogenesis
Gap junction alpha-1 protein (connexin 43)	GJA1	Ssc.29187.1.A1_at	201667_at	Cellular morphogenesis during differentiation Neuron morphogenesis during differentiation Neurite morphogenesis Neuron development Cell projection morphogenesis- and organization Cell part morphogenesis Generation of neurons and neuron differentiation Neurogenesis Neurite development Cell- and cell structure morphogenesis
GATA binding protein 2	GATA2	Ssc.4743.1.S2_at	210358_x_at	Generation of neurons and neuron differentiation Neurogenesis
Gli-kruppel family member gli2	GLI2	Ssc.2318.1.A1_at	228537_at	Cellular morphogenesis during differentiation Axonogenesis Neuron morphogenesis during differentiation Neurite morphogenesis Neuron development Cell projection morphogenesis- and organization Cell part morphogenesis Generation of neurons and neuron differentiation Neurogenesis

(*Continued*)

Table 5 Continued

Annotation cluster 21: Neurogenesis-relevant gene ontology (GO) terms		Affymetrix ID		
Gene name	Gene ID	Porcine	Human	GO terms
				Neurite development
				Cell- and cell structure morphogenesis
Gli-kruppel family member gli3	GLI3	Ssc.9998.1.A1_at	205201_at	Generation of neurons and neuron differentiation
				Neurogenesis
Insulin-like growth factor binding protein 5	IGFBP5	Ssc.15800.1.S1_at	211958_at	Cell- and cell structure morphogenesis
Integrin alpha 8	ITGA8	Ssc.1377.1.A1_at	214265_at	Cell projection morphogenesis
				Cell part morphogenesis- and organization
				Cell- and cell structure morphogenesis
Laminin beta 1	LAMB1	Ssc.24580.1.A1_at	211651_s_at	Neuron development
				Cell projection morphogenesis- and organization
				Cell part morphogenesis
				Generation of neurons and neuron differentiation
				Neurogenesis
				Neurite development
				Cell- and cell structure morphogenesis
Matrix metallopeptidase 14	MMP14	Ssc.734.1.S1_at	202828_s_at	Neurogenesis
Microtubule associated protein 1b	MAP1B	Ssc.21472.2.A1_at	226084_at	Neuron development
				Cell projection morphogenesis- and organization
				Cell part morphogenesis
				Generation of neurons and neuron differentiation
				Neurogenesis
				Neurite development
				Cell- and cell structure morphogenesis
Myosin, heavy polypeptide 10	MYH10	Ssc.20017.3.A1_at	213067_at	Cell- and cell structure morphogenesis
Natriuretic peptide receptor A	NPR1	Ssc.24926.1.S1_at	32625_at	Cell- and cell structure morphogenesis

Necdin homolog	NDN	Ssc.7155.1.A1_at	209550_at	Cellular morphogenesis during differentiation Axonogenesis Neuron morphogenesis during differentiation Neurite morphogenesis Neuron development Cell projection morphogenesis- and organization Cell part morphogenesis Generation of neurons and neuron differentiation Neurogenesis Neurite development Cell- and cell structure morphogenesis
Neurogenic Notch homolog 3	NOTCH3	Ssc.3778.1.S1_at	203238_s_at	Generation of neurons and neuron differentiation Neurogenesis
Nuclear receptor subfamily 2, group f, member 1	NR2F1	Ssc.28190.1.A1_at	209505_at	Generation of neurons Neurogenesis
Par-3 partitioning defective 3 homolog	PARD3	Ssc.5238.1.S1_at	210094_s_at	Cellular morphogenesis during differentiation Axonogenesis Neuron morphogenesis during differentiation Neurite morphogenesis Neuron development Cell projection morphogenesis- and organization Cell part morphogenesis Generation of neurons and neuron differentiation Neurogenesis Neurite development Cell- and cell structure morphogenesis
Phosphatidylinositol-4-phosphate 5-kinase, type I, gamma	PIP5K1C	Ssc.26082.2.S1_at	212518_at	Cellular morphogenesis during differentiation Axonogenesis Neuron morphogenesis during differentiation Neurite morphogenesis Neuron development

(*Continued*)

Table 5 Continued

Annotation cluster 21: Neurogenesis-relevant gene ontology (GO) terms		Affymetrix ID		
Gene name	Gene ID	Porcine	Human	GO terms
				Cell projection morphogenesis- and organization Cell part morphogenesis Generation of neurons and neuron differentiation Neurogenesis Neurite development Cell- and cell structure morphogenesis
Proto-oncogene tyrosine-protein kinase	FYN	Ssc.2714.3.S1_at	212486_s_at	Generation of neurons Neurogenesis Cell- and cell structure morphogenesis
Putative Rho/Rac guanine nucleotide exchange factor	FGD1	Ssc.6359.1.A1_at	204819_at	Cell projection morphogenesis- and organization Cell part morphogenesis Cell- and cell structure morphogenesis
Ras p21 protein activator	RASA1	Ssc.7894.1.A1_at	202677_at	Cell- and cell structure morphogenesis
Ring finger protein 6	RNF6	Ssc.29730.1.A1_at	210932_s_at	Cellular morphogenesis during differentiation Axonogenesis Neuron morphogenesis during differentiation Neurite morphogenesis Neuron development Cell projection morphogenesis- and organization Cell part morphogenesis Generation of neurons and neuron differentiation Neurogenesis Neurite development Cell- and cell structure morphogenesis
Sphingolipid G-protein coupled receptor 1	EDG1	Ssc.23526.1.S1_at	204642_at	Generation of neurons and neuron differentiation Neurogenesis
Tenascin N	TNN	Ssc.5738.1.A1_at	215271_at	Cellular morphogenesis during differentiation Axonogenesis

				Neuron morphogenesis during differentiation Neurite morphogenesis Neuron development Cell projection morphogenesis- and organization Cell part morphogenesis Generation of neurons and neuron differentiation Neurogenesis Neurite development Cell- and cell structure morphogenesis
Thy-1 cell surface antigen	THY1	Ssc.6634.2.S1_at	208850_s_at	Cellular morphogenesis during differentiation Axonogenesis Neuron morphogenesis during differentiation Neurite morphogenesis Neuron development Cell projection morphogenesis- and organization Cell part morphogenesis Generation of neurons and neuron differentiation Neurogenesis Neurite development Cell- and cell structure morphogenesis
Transforming growth factor beta 2 (TGF-beta 2)	TGFB2	Ssc.12131.1.A1_at	220407_s_at	Cellular morphogenesis during differentiation Axonogenesis Neuron morphogenesis during differentiation Neurite morphogenesis Neuron development Cell projection morphogenesis- and organization Cell part morphogenesis Generation of neurons and neuron differentiation Neurogenesis Neurite development Cell- and cell structure morphogenesis
Transforming growth factor beta 3	TGFB3	Ssc.27593.1.S1_at	209747_at	Cell- and cell structure morphogenesis

(*Continued*)

Table 5 Continued

Annotation cluster 21: Neurogenesis-relevant gene ontology (GO) terms		Affymetrix ID		
Gene name	Gene ID	Porcine	Human	GO terms
(TGF-beta 3)				
Twist homolog 1	TWIST1	Ssc.24416.1.S1_at	213943_at	Generation of neurons Neurogenesis
Ubiquitin thiolesterase	UCHL1	Ssc.2073.1.A1_at	201387_s_at	Cellular morphogenesis during differentiation Axonogenesis Neuron morphogenesis during differentiation Neurite morphogenesis Neuron development Cell projection morphogenesis- and organization Cell part morphogenesis Generation of neurons and neuron differentiation Neurogenesis Neurite development Cell- and cell structure morphogenesis
Vinculin	VCL	Ssc.11071.2.S1_at	200931_s_at	Cell projection morphogenesis- and organization Cell part morphogenesis Cell- and cell structure morphogenesis
Voltage-dependent L-type calcium channel alpha-1 F subunit	CACNA1 F	Ssc.13160.1.A1_at	208377_s_at	Cellular morphogenesis during differentiation Axonogenesis Neuron morphogenesis during differentiation Neurite morphogenesis Neuron development Cell projection morphogenesis- and organization Cell part morphogenesis Generation of neurons and neuron differentiation Neurogenesis Neurite development Cell- and cell structure morphogenesis

Table 6 Consistently expressed genes relevant for cell signaling processes as identified by the DAVID functional annotation analysis of the swine neuronal OBGF400 cells

Annotation cluster 24: Cell signaling-relevant gene ontology (GO) terms		Affymetrix ID		
Gene name	Gene ID	Porcine	Human	GO terms
Acetylcholine receptor protein, alpha chain	CHRNA1	Ssc.19688.1.S1_at	206633_at	Cell-cell signaling Transmission of nerve impulse Synaptic transmission Neurological system process System process
Adrenergic alpha-1B receptor	ADRA1B	Ssc.17370.1.A1_at	207589_at	Cell-cell signaling Neurological system process System process
Amphiphysin	AMPH	Ssc.5290.1.S1_at	205257_s_at	Cell-cell signaling Transmission of nerve impulse Synaptic transmission Neurological system process System process
Barh-like homeobox	BARX1	Ssc.27415.1.S1_at	219845_at	Cell-cell signaling
Beta-neoendorphin-dynorphin (proenkephalin B)	PDYN	Ssc.121.1.S1_at	206803_at	Cell-cell signaling Transmission of nerve impulse Synaptic transmission Neurological system process System process
Calponin 1	CNN1	Ssc.9013.1.S1_at	203951_at	System process
Calponin 3	CNN3	Ssc.1745.1.A1_at	201445_at	System process
Chemokine ligand 6	CXCL6	Ssc.719.1.S1_a_at	206336_at	Cell-cell signaling
Collagen alpha 1 chain	COL5A2	Ssc.1091.2.S1_at	202312_s_at	Neurological system process System process
Collagen alpha 1(III) chain	COL3A1	Ssc.11302.1.S2_at	215076_s_at	System process
Collagen alpha 2 chain	COL1A2	Ssc.9362.1.A1_at	202404_s_at	Neurological system process

(*Continued*)

Table 6 Continued

Annotation cluster 24: Cell signaling-relevant gene ontology (GO) terms		Affymetrix ID		
Gene name	Gene ID	Porcine	Human	GO terms
Contactin associated protein-like 2	CNTNAP2	Ssc.27213.1.A1_at	219302_s_at	System process Cell-cell signaling Transmission of nerve impulse Neurological system process System process
Cytochrome P450 1B1	CYP1B1	Ssc.12616.1.A1_at	208807_s_at	Neurological system process System process
Drebrin 1 (developmentally regulated brain protein)	DBN1	Ssc.30502.1.A1_at	202806_at	Cell-cell
signaling				Transmission of nerve impulse Synaptic transmission Neurological system process System process
Dystroglycan 1	DAG1	Ssc.27536.1.S1_at	212128_s_at	System process
Dystrophin	DMD	Ssc.27279.1.S1_at	208086_s_at	System process
EGF-containing fibulin-like extracellular matrix protein 1	EFEMP1	Ssc.20515.1.S1_at	201842_s_at	Neurological system process System process
Endothelin A receptor	EDNRA	Ssc.16189.1.S1_at	204463_s_at	System process
Ephrin-B2	EFNB2	Ssc.27799.1.S1_at	202669_s_at	Cell-cell signaling
ERGB transcription factor	FLI1	Ssc.2368.1.S1_at	211825_s_at	System process
Excitatory amino acid transporter 1 (glutamate transporter)	SLC1A3	Ssc.8447.1.A1_at	202800_at	Cell-cell signaling Transmission of nerve impulse Synaptic transmission Neurological system process System process
Fibroblast growth factor-12	FGF12	Ssc.30632.1.S1_at	207501_s_at	Cell-cell signaling
Fibroblast growth factor-13	FGF13	Ssc.5107.1.A1_at	205110_s_at	Cell-cell signaling
Follistatin	FST	Ssc.4747.1.S1_at	226847_at	Cell-cell signaling
Forkhead box c2	FOXC2	Ssc.19569.1.S1_at	214520_at	System process
Frizzled-1	FZD1	Ssc.28993.1.S1_at	204452_s_at	Cell-cell signaling

Fxyd domain containing ion transport regulator 1	FXYD1	Ssc.5133.1.S1_at	205384_at	System process
Gamma-aminobutyric acid type B receptor, subunit 2	GPR51	Ssc.2436.1.A1_at	211679_x_at	Cell-cell signaling Transmission of nerve impulse Synaptic transmission Neurological system process System process
Gap junction beta-2 protein (connexin 26)	GJB2	Ssc.19122.1.A1_at	223278_at	Cell-cell signaling Neurological system process System process
Gap junction alpha-1 protein (connexin 43)	GJA1	Ssc.29187.1.A1_at	201667_at	Cell-cell signaling Neurological system process System process
GATA binding protein 3	GATA3	Ssc.27964.2.S1_at	209604_s_at	Cell-cell signaling Transmission of nerve impulse Synaptic transmission Neurological system process System process
Glutamate receptor, ionotropic kainate 2	GRIK2	Ssc.4849.1.A1_at	213845_at	Cell-cell signaling Transmission of nerve impulse Synaptic transmission Neurological system process System process
Glutamate [NMDA] receptor subunit epsilon 2 precursor	GRIN2B	Ssc.19028.1.A1_at	210412_at	Cell-cell signaling Transmission of nerve impulse Synaptic transmission Neurological system process System process
Hemicentin	NP_114141	Ssc.26893.1.A1_at	235944_at	Neurological system process System process
Insulin-like-growth factor 1 (somatomedin C)	IGF1	Ssc.12578.1.A1_at	209542_x_at	Neurological system process System process
Latexin	LTX	Ssc.2983.1.A1_at	218729_at	Neurological system process System process
Lipophilin	PLP1	Ssc.8177.1.A1_at	210198_s_at	Cell-cell signaling

(*Continued*)

Table 6 Continued

Annotation cluster 24: Cell signaling-relevant gene ontology (GO) terms		Affymetrix ID		
Gene name	Gene ID	Porcine	Human	GO terms
				Transmission of nerve impulse Synaptic transmission Neurological system process System process
Lumican	LUM	Ssc.1128.1.S1_at	229554_at	Neurological system process System process
Matrix Gla-protein	MGP	Ssc.373.1.S1_at	202291_s_at	Neurological system process System process
Monoamine oxidase A	MAOA	Ssc.23505.1.S1_at	212741_at	Cell-cell signaling Transmission of nerve impulse Synaptic transmission Neurological system process System process
Muscarinic acetylcholine receptor M2	CHRM2	Ssc.9858.1.A1_at	221330_at	System process
Myoferlin	FER1L3	Ssc.18040.1.A1_at	201798_s_at	System process
Myomesin 1	MYOM1	Ssc.19335.1.S1_at	205610_at	System process
Myosin, heavy polypeptide 11	MYH11	Ssc.10392.1.A1_at	207961_x_at	System process
Myosin binding protein H	MYBPH	Ssc.20879.1.S1_at	206304_at	System process
Natriuretic peptide receptor A	NPR1	Ssc.24926.1.S1_at	32625_at	System process
Natriuretic peptide receptor C	NPR3	Ssc.9019.1.A1_at	219790_s_at	System process
Natriuretic peptide receptor CB	NPR2	Ssc.25297.1.S1_at	204310_s_at	System process
Necdin homolog	NDN	Ssc.7155.1.A1_at	209550_at	Neurological system process System process
Neural cell adhesion molecule 1	NCAM1	Ssc.6092.1.A1_at	209968_s_at	Cell-cell signaling Transmission of nerve impulse Synaptic transmission Neurological system process System process
Neuregulin 1	Nrg1	Ssc.4398.1.S1_at	206343_s_at	Cell-cell signaling

				Transmission of nerve impulse
				Neurological system process
				System process
Neuropeptide Y	NPY	Ssc.15981.1.S1_at	206001_at	Cell-cell signaling
				Transmission of nerve impulse
				Synaptic transmission
				Neurological system process
				System process
Neuropeptide Y receptor type 1	NPYR1	Ssc.16348.1.S1_at	205440_s_at	Neurological system process
				System process
Paired box protein Pax-3	PAX3	Ssc.27600.1.S1_at	231666_at	Neurological system process
				System process
Peripheral myelin protein 22	PMP22	Ssc.11108.1.A1_at	210139_s_at	Cell-cell signaling
				Transmission of nerve impulse
				Synaptic transmission
				Neurological system process
				System process
Phosphodiesterase 4d, cAMP specific	PDE4D	Ssc.17233.1.A1_at	204491_at	System process
Phytanoyl-CoA-2-hydroxylase	PHYH	Ssc.8139.1.S1_at	203335_at	Neurological system process
				System process
Potassium large conductance calcium activated channel beta	KCNMB1	Ssc.10064.2.A1_at	209948_at	Cell-cell signaling
				Transmission of nerve impulse
				Synaptic transmission
				Neurological system process
				System process
Potassium voltage-gated channel, subfamily h, member 2	KCNH2	Ssc.30718.1.A1_at	205262_at	Neurological system process
				System process
Proprotein convertase subtilisin/kexin type 5	PCSK5	Ssc.10078.1.A1_at	205559_s_at	Cell-cell signaling
Prostaglandin-endoperoxide synthase 2	PTGS2	Ssc.23994.1.A1_at	1554997_a_at	System process
Regulating synaptic membrane exocytosis protein 1	RIMS1	Ssc.4379.1.A1_at	216184_s_at	Cell-cell signaling
				Transmission of nerve impulse
				Synaptic transmission
				Neurological system process

(*Continued*)

Table 6 Continued

Annotation cluster 24: Cell signaling-relevant gene ontology (GO) terms		Affymetrix ID		
Gene name	Gene ID	Porcine	Human	GO terms
				System process
Sarcospan	SSPN	Ssc.25882.1.S1_at	204963_at	System process
Serotonin receptor 2A	HTR2A	Ssc.16141.1.S1_at	211616_s_at	Cell-cell signaling Transmission of nerve impulse Synaptic transmission Neurological system process System process
Serotonin receptor 2C	HTR2C	Ssc.15372.1.A1_at	211479_s_at	Cell-cell signaling Transmission of nerve impulse Synaptic transmission Neurological system process System process
Small inducible cytokine B14 precursor	CXCL14	Ssc.20578.1.S1_at	218002_s_at	Cell-cell signaling
Sodium-dependent glutamate/aspartate transporter	SLC1A6	Ssc.4979.1.S1_at	206882_at	Cell-cell signaling Transmission of nerve impulse Synaptic transmission Neurological system process System process
Sphingolipid G-protein coupled receptor 1	EDG1	Ssc.23526.1.S1_at	204642_at	Cell-cell signaling Transmission of nerve impulse Neurological system process System process
Stanniocalcin 1	STC1	Ssc.15105.1.S1_at	204596_s_at	Cell-cell signaling
T-box 3	TBX3	Ssc.28678.1.S1_at	225544_at	Cell-cell signaling
Titin	TTN	Ssc.10947.1.S1_at	208195_at	System process
Thyroid hormone receptor beta-1	THRB	Ssc.26200.1.S1_at	207044_at	Neurological system process System process
Transforming growth factor beta 2 (TGF-beta 2)	TGFB2	Ssc.12131.1.A1_at	220407_s_at	Cell-cell signaling Transmission of nerve impulse

				Synaptic transmission Neurological system process System process
Transforming growth factor beta 3	TGFB3	Ssc.27593.1.S1_at	209747_at	Cell-cell signaling
Tropomyosin	TPM1	Ssc.12185.1.A1_at	206117_at	System process
Troponin C type 1	TNNC1	Ssc.19566.1.S1_at	209904_at	System process
Tumor necrosis factor alpha-induced protein 6	TNFAIP6	Ssc.30887.1.S1_at	206025_s_at	Cell-cell signaling
Tumor necrosis factor ligand superfamily member 4	TNFSF4	Ssc.23487.1.S1_at	207426_s_at	Cell-cell signaling
Voltage-dependent N-type calcium channel alpha-1B subunit	CACNA1B	Ssc.19324.1.A1_at	207162_s_at	Cell-cell signaling Transmission of nerve impulse Synaptic transmission Neurological system process System process
Voltage-dependent L-type calcium channel alpha-1C subunit	CACNA1C	Ssc.4599.1.A1_at	208020_s_at	System process
Voltage-dependent L-type calcium channel alpha-1F subunit	CACNA1F	Ssc.13160.1.A1_at	208377_s_at	Cell-cell signaling Transmission of nerve impulse Synaptic transmission Neurological system process System process

1 (DBN1), myomesin (MYOM1), titin (TTN), myoferlin (FER1L3), as well as the myosin heavy polypeptide 11 (MYH11) and the myosin binding protein H (MYBPH), were categorized under the GO term system process (cluster 24) and not directly linked to the tangential migration of SVZ/OB-derived neuronal progenitors. However, directly associated with neuronal development and differentiation was the cAMP responsive element binding protein 1 (CREPB1) as well as the transforming growth factor beta 2 (TGFB2). Cell cycle regulators included the cyclin-dependent kinase inhibitors 2A (p16, CDKN2A) and 1A (p21), with the latter not being represented on the list of 831 probe sets submitted for the DAVID functional annotation analysis.

As listed in Table 6, several detected gene products relevant for synaptic transmission and neurological system processes defined a gamma aminobutyric acid (GABA)-ergic and glutamatergic phenotype for the OBGF400 cells. In addition, the identified expression of the G-protein-coupled GABA type B receptor subunit 2 (GPR51), as well as the transcription of the excitatory amino acid transporter 1 (glutamate transporter 1, SLC1A3), the ionotropic glutamate receptor kainate 2 (GRIK2), and the N-methyl-D-aspartate receptor (NMDAR) subunit epsilon (GRIN28), prompted a more detailed analysis of the gene products associated with either the chief inhibitory neurotransmitter GABA or the excitatory neurotransmitter glutamate. Although not represented on the list of the unique 831 probe sets, yet identified as consistently transcribed by OBGF400 cells, were the GABA receptor gamma-1 and alpha-5 subunits, the ion channel GABA type A receptor-associated proteins 1 and 2, the glutamate transporter 5, sodium-dependent glutamate/aspartate transporters 1 and 6, as well as the synaptic vesicular monoamine transporter with the ionotropic glutamate receptor α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA)1 genes. In agreement with the GABAergic nature of the OBGF400 cells, the necdin gene (NDN) expression product, predominantly expressed in mature neurons and known to facilitate the specification of GABAergic neurons (15), was identified in the neuroblasts. The NDN homolog also was allocated to the biological processes of neurogenesis and neuron differentiation.

Identification of transcripts for the neuropeptide Y (NPY) and its corresponding receptor (NPYR1) suggested olfactory neuromodulatory activities in the immortalized neuroblasts. Additionally, the coexistence of the two distinct serotonin receptors 2A (HTR2A) and 2C (HTR2C) extended the complexity of the neurotransmitter system in the committed precursors. The monoamine oxidase A (MAOA), which is vital for the inactivation of monoaminergic neurotransmitters such as serotonin, was also expressed in the OBGF400 neuroblasts.

The exocytotic machinery essential for neurotransmission was characterized in the porcine neuroblasts by the detection of the transcript for the regulating synaptic membrane exocytosis protein 1. This gene expression product was assigned GO terms of transmission of nerve impulse, synaptic transmission, and neurological system process. The additional presence of mRNA for vesicle-associated Ca^{2+}-binding protein synaptotagmin I, syntaxin 16, synaptosomal-associated proteins (SNAPs) of 23 kDa and 29 kDa, synaptic membrane vesicle amine transport protein 1 homolog, regulating synaptic membrane exocytosis protein 2, as well as synaptobrevin (not represented on the list submitted for DAVID functional annotation analysis),

enabled further definition of the intercellular trafficking of proteins leading up to membrane fusion and exocytosis of neurotransmitters within the OBGF400 cells.

The multiplicity of ion channels detected in the transcriptional profile of the neuroblasts was noteworthy. Alpha-1B, −1C, −1F subunits of the voltage dependent L-type and N-type calcium channels, both of which fall into the group of high voltage-activated calcium channels, were categorized. Additionally, a multitude of channels relevant for ionic transport were recognized and included Ca^{2+}-activated potassium channel beta and a voltage-gated potassium channel of the subfamilies H.

DISCUSSION

Mitotic competency in morphologically mature neurons of the CNS is considered contrary to the central paradigm that neuronal cells become postmitotic upon maturation-induced, terminal differentiation. Intriguingly, OB-derived, neuronal lineage-committed precursor cells retain their ability to actively divide while simultaneously displaying certain characteristics of a mature phenotype. In an effort to establish a continuous source of such neuronal cells, we previously immortalized porcine OB-derived neuroblasts through the direct insertion of the catalytic subunit of the hTERT cDNA into their chromosomes, yielding the OBGF400 cell line (1). Contrary to the standard oncogene-dependent retroviral immortalization techniques, ectopic over-expression of hTERT generally does not result in neoplastic transformation of cultured cells (16, 17). Although numerical and structural chromosome anomalies can follow ectopic telomerase expression (18), the occurrence of such cancer-associated changes is rare and generally accompanied by anchorage independent growth. The cultured OBGF400 cells maintained contact inhibition and anchorage dependent growth. These observations, coupled with the stable gene expression of tumor suppressor protein p53 and proto-oncogene c-myc, provided further evidence that cellular transformation events had not occurred in the immortalized neuroblasts.

Another held tenet maintains that aneuploid neuroblasts are destined to undergo cell death. Although chromosomal segregation defects in neuronal progenitor cells promote the generation of aneuploid neuroblasts (19), it now has been established that a significant population of neuronal cells displaying such abnormal chromosomal complements not only survive, but are functionally integrated into the intricate neural networks (5, 20). Indeed, non-homologous end joining and homologous recombination are both essential mechanisms during brain development (21). Such numerical anomalies have been identified in viable neuronal precursors in the postnatal neurogenic SVZ and the OB, both in *in vivo* and *in vitro* contexts, and were attributed to chromosomal segregation defects such as non-disjunction, multipolar, or bipolar division and lagging chromosomes. Consequently, the generation of aneuploid neurons, rather than being detrimental, creates a unique genetic diversity or mosaic in the neural network (4, 19). The resultant endogenous gene expression patterns thereby establish genetic imprinting and allelic inactivation (20, 22). Therefore, not unexpectedly, the metaphase profile of the OBGF400 cells revealed hyperdiploidy.

Previous attempts utilizing porcine-specific expressed sequence tags to define neuron-associated genes were limited based on the fact that whole tissue was used

rather than isolated neurons (23, 24). The relatively new Affymetrix Gene Chip® Porcine Genome Array represents 24,123 probe sets of *Sus scrofa* genes, while the new annotation file from Tsai et al. (7) contains 12,124 unique genes. Employment of this array and annotation file enabled the identification of the expression program in the OBGF400 cells and, thereby, delineated the first whole genome-scaled transcriptome analysis of this unique population of committed porcine neuronal precursors. A total of 12,867 probe sets corresponding to 8,287 genes (7) were identified as being hybridized by the OBGF400 neuroblast transcripts.

Although microarray technologies have greatly improved in their performance, the accuracy of the detection calls is still a frequent subject of discussion. There was a stringent consistency among our three independent biological replicates from which we were able to determine that only 9.8% identified gene expression products were expected to be false positives. As mere transcription of an encoded gene does not necessarily guarantee its protein synthesis, extrapolation from gene expression information to the protein level must be drawn with considerable caution. Nevertheless, this study significantly broadened the understanding of the neuronal phenotype of the OBGF400 cells and presented remarkably coherent signaling patterns that most likely are applicable in broad parts to the *in vivo* situation of these postnatal OB precursors. Although the lack of extrinsic cues and intercellular signaling during *in vitro* cultivation of progenitor cells certainly alters intrinsic biological themes, several biological processes relevant for the creation of the genetic mosaicism, neurogenesis and impulse transmission were recognized and revealed the previously unreported signaling dynamics of these neuroblasts.

Indisputably, the generation of genomic diversity is crucial for the complexity of the CNS. Some molecules involved in the regulation of neurogenesis also participate in neoplastic transformation. As disclosed by the expression profile, several of these genes were constitutively expressed in the cultured OBGF400 cells and included non-homologous end joining factors (XRCC4, Ku70, and Ku80), DNA ligase IV and CIB1, all of which function in DNA double strand repair mechanisms (25). Deficiencies in these factors during nervous system development may result in premature cellular senescence and apoptosis of newly formed neurons. In addition, the interplay between the proteins of the pituitary tumor transforming 1 (securin) and the extra spindle pole like 1 (separanase) genes (both transcribed in the OBGF400 cells) is critical for the cleavage of sister chromatids during the metaphase to anaphase transition (26) and thus, their absence or inactivation may contribute to neuronal aneuploidy. Moreover, neurons with numerical chromosomal instability, such as the OBGF400 cells, rather than undergoing cell death during neurogenesis, may actually participate in active neuronal signaling. A study by Kingsbury et al. (5) demonstrated that neurons with such karyotypic abnormalities were functionally integrated into neuronal circuitries by revealing through nuclear immunolabeling the presence of the immediate early gene (IEG) products c-Fos and early growth response protein 1 (EGR-1). Since IEG induction is believed to coincide with neuronal stimulation and changes in synaptic plasticity (27), IEG proteins have been widely applied as markers of neuronal activity and synaptic stimulation (28). The transcriptome profile of OBGF400 cells revealed the expression of several IEGs: EGR-1, FOS, JUN, and JUNB.

Similarly, the expression profile established the GABA and glutamatergic character of the OBGF400 cells. Tangentially migrating neuroblasts derived from

the SVZ/RMS first predominantly express extrasynaptic GABA type A receptors prior to the synthesis of AMPA receptors. As the neurons mature in the more peripheral layers of the OB, glutamate-responding NMDAR is expressed (29). In accordance with these findings, several GABA receptor subunits and GABA type A-associated protein transcripts were identified in the cultured OBGF400 cells. Also transcribed were the genes for the glutamate receptors AMPA1 and kainate 2 as well as the NMDAR subunit. Within a similar context, the expression of necdin, a member of the melanoma antigen protein family, recently has been shown to augment the differentiation and specification of GABAergic neurons (15). In addition to the GABA- and glutamatergic character of the OBGF400 cells, the serotonergic system modulates the olfactory processing system (30). Serotonin selectively influences signal transduction via classical synaptic stimulation or non-junctional transmission and serotonin neurons often coexist with neuropeptides (31); OBGF400 neuroblasts expressed the serotonin receptors 2A and 2C concurrently with the neuropeptide Y, a modulator of excitatory synaptic transmission in the OB (32). The exact extent and participation of the OBGF400 cells in neurotransmitter-mediated signal transduction through functional and integrated GABA, glutamate, and serotonin receptors remains to be verified.

Another important facet of signal transmission in neuronal cells is the ability to exert membrane potentials and trigger calcium influx. Neuronal calcium acts not only as a charge carrier, but also enables information processing in its role as a ubiquitous intracellular messenger. The functional annotation identified a rich cluster of ion channels including high voltage-gated N- and L-type calcium channels as well as potassium and sodium channels. The Ca^{2+} ions that enter by the L-type channels are implicated in a variety of physiological cell processes, such as the regulation of gene expression and excitation-induced neurotransmission. The N-type channels, however, are primarily located at presynaptic terminals and bind syntaxin, a protein that is localized in the immediate vicinity to the neuronal plasma membrane (33). The genes for syntaxin I, synaptotagmin I, synaptobrevin, SNAPs precursors 23–29 and vesicle amine transport protein 1 (a synaptic vesicle membrane protein) were transcribed in the OBGF400 cells. Their respective proteins play a fundamental role in the molecular events often referred to as the "SNARE hypothesis" that leads to vesicle docking and membrane fusion during neurotransmitter release (34–36). As a Ca^{2+} sensor, synaptotagmin I is essential for the temporal coupling between Ca^{2+} influx and synaptic vesicle fusion (37).

Unquestionably, signal transduction patterns play an exceedingly crucial role in the neurogenic processes occurring in progenitor cells. It is considered that these hTERT-immortalized neuroblasts sustain their mitotic competency through continuous telomere extension as once their overall net telomerase activity decreases; the OBGF400 cells morphologically change into mature neurons as indicated by the formation of multipolar processes and a more defined cell body (1). Although the exact mechanism(s) underlying the transition of the immortalized neuroblasts into cellular senescence have yet to be determined, the functional annotation revealed the involvement of several expressed genes in cellular differentiation, neurite outgrowth and axonogenesis, which included: NRCAM, DBN1, and TGFB2. Whereas complex formation of neuronal cell adhesion molecule (NRCAM) with contactin induces neurite growth and neuronal differentiation (38), drebrin 1 is a major F-actin binding

protein involved in the control of actin dynamics and neuronal morphogenesis (39). Several other expression products involved in the regulation of cytoskeletal dynamics, neurite outgrowth or cell migrations were identified. Members of the tenascin family, represented by tenascin N in the OBGF400 cells, mediate neurite development and neuroblast migration (40, 41). Neuroblast migration along the RMS requires so-called serum response factors (42). These factors activate target genes that encode cytoskeletal proteins such as myosin and vinculin, both consistently transcribed by the OBGF400 cells. Integrin signaling further controls axonal guidance cues and axonal patterning, thereby contributing to the formation of neural networks (43). Integrin alpha 8 as well as the axon guidance molecule ephrin-B2 were expressed in the immortalized neuroblasts. Although the role of TGF-beta in neuronal progenitor cells and mature neurons is diverse, it is known to participate in neuronal differentiation and stimulate *in vitro* neurite outgrowth of primary neurons (44, 45). TGF-beta signaling, in collaboration with Smad complexes, elicits G1 cell cycle arrest by the activation of the candidate tumor suppressor gene product p15. The p15 protein inhibits synthesis of cyclin-dependent kinase 4 (CDK4) (46) and, thus, interferes with the ability of this kinase to advance cell cycle progression. Although p15 was not expressed in the OBGF400 cells, its functional correspondents p16 (CDKN2A) and p21 (CDKN1A) were present in the neuroblasts. Similar to p15, p16 and p21 inhibit CDK4 and, hence, impede G1 phase progression (47). This supported the finding that OBGF400 cells are predominately in the GO/G1 phase (1).

Overall, the identified genomic complexity of the OBGF400 cells provided an in-depth insight into the elaborate signaling mechanisms of this unique subpopulation of porcine neuronally committed progenitors and clearly mirrors the intricate organization of postnatal neurogenic zones. By virtue of its genomic diversity, its innate transcription pattern, its neuronal phenotype, and the lack of evidence of neoplastic transformation, the OBGF400 cell line offers a novel and useful *in vitro* system to facilitate the examination of neuronal signaling patterns. In addition, this cell line will enable the *in vitro* exploration of neuronal cell-pathogen interactions and thus aid in the advancement of infectious disease investigations.

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